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Relationship of glucocorticoids and hematological measures with feed intake, growth, and efficiency of finishing beef cattle[1]

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ABSTRACT: The objective of this experiment was to determine the association of glucocorticoids and markers for immune status in finishing beef steers and heifers with DMI, growth, and efficiency. Steers (n = 127) and heifers (n = 109) were individually fed a finishing ration for 84 d with BW measured every 21 d. Blood samples were collected via jugular venipuncture for metabolite (glucose and lactate) and cortisol analysis and rectal grab samples of feces were collected for corticosterone analysis on d 83 of the experiment. Plasma cortisol was not correlated to DMI (r = –0.08, P > 0.05) or fractional DMI (g DMI/kg BW; r = –0.03, P > 0.05) but was negatively correlated with ADG (r = –0.17, P < 0.01) and G:F (r = –0.20, P < 0.01) and positively correlated to residual feed intake (RFI; r = 0.14, P < 0.05). Fecal corticosterone was positively correlated to fractional DMI (r = 0.15, P < 0.05) and RFI (r = 0.23, P < 0.01) and negatively correlated to G:F (r = –0.18, P < 0.01). Using a mixed model analysis, none of the metabolites or hormones were associated with DMI (P > 0.05) but fecal corticosterone was positively associated with fractional DMI only in heifers (P = 0.04). Plasma lactate (P < 0.01) was and plasma cortisol (P < 0.10) tended to be negatively associated with ADG. Plasma cortisol (P < 0.05) and fecal corticosterone tended (P < 0.10) to be negatively associated with G:F. Fecal corticosterone was positively associated with RFI in heifers (P < 0.04). In a mixed model analysis, total leukocyte count was positively associated with ADG (P < 0.04) and tended to be positively associated with G:F (P < 0.06). Among leukocyte subtypes, neutrophil count was positively associated with ADG in steers (P < 0.02) and monocytes were positively associated with ADG in heifers (P < 0.03). Lymphocyte counts (LY) in steers were negatively associated with DMI (P = 0.03) and fractional DMI (P < 0.03). In heifers, LY tended to be positively associated with DMI (P < 0.09) and fractional DMI (P < 0.06). Lymphocyte count was also positively associated with ADG (P < 0.01) and G:F (P = 0.05) in heifers. The association of production traits with immune status seems to be different between steers and heifers. There was a stronger relationship of cortisol than fecal corticosterone to feed efficiency measures, suggesting that cortisol concentrations could be a better marker for feed efficiency traits than fecal corticosterone concentrations.

Key words: feed efficiency, glucocorticoids, hematology, physiological markers

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INTRODUCTION

Recent feed efficiency research has focused on discovering physiological markers for feed efficiency in beef cattle. To improve the feed efficiency of beef cattle without having deleterious effects on other traits, it is necessary to find specific selection criteria other than a broad trait such as ADG, as selecting for growth leads to cattle that have a greater mature BW (Koots et al., 1994) and, therefore, increases maintenance requirements for the maternal portion of the industry. Other criteria such as residual feed intake (**RFI**) have proven

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problematic in cattle and other species (Pitchford, 2004) and leads to less active animals and reproductive problems (Pitchford, 2004; Wang et al., 2012). It may be possible to find physiological mechanisms that are individually associated with feed intake, growth, or actual feed efficiency yet not be related to mature body size, activity, or reduced reproductive efficiency.

Two potential physiological mechanisms that could be associated with feed efficiency are immune function and glucocorticoid concentrations. There is no doubt that disease in livestock leads to reduced growth and feed efficiency (Johnson, 1997; Spurlock, 1997), but it is also possible that subclinical disease or the ability to defend against disease could be associated with efficient G:F. Additionally, variation in glucocorticoid concentrations can lead to inefficient use of vital nutrients (Baxter and Forsham, 1972), which would likely lead to poor growth and nutrient conversion. The objectives of this experiment were to determine the association of glucocorticoid concentrations and measures of immune status with feed intake, growth, and feed efficiency of finishing beef cattle. It is hypothesized that increases in immune function and glucocorticoid concentrations will be associated with a decrease in feed efficiency.

MATERIALS AND METHODS

This experiment was reviewed and approved by the U.S. Meat Animal Research Center Animal Care and Use Committee (Institutional Animal Care and Use Committee [**IACUC**] approval number 5438-31000-0092-03) under IACUC standards meeting the requirements outlined by the USDA.

Calves (n = 236) were from composite breed cows (MARC I [one-fourth Limousin, one-fourth Charolais, one-fourth Braunvieh, one-eighth Hereford, and one-eighth Angus], MARC II [one-fourth Simmental, one-fourth Gelbvieh, one-fourth Hereford, and one-fourth Angus], and MARC III [one-fourth Pinzgauer, one-fourth Red Poll, one-fourth Hereford, and one-fourth Angus]) mated to Angus (n = 9), Charolais (n = 4), Gelbvieh (n = 7), Limousin (n = 5), Red Angus (n = 4), and Simmental (n = 7) bulls that were in current use on industry ranches. Both steers (sired by Angus [n = 35], Charolais [n = 8], Gelbvieh [n = 21], Limousin [n = 21], Red Angus [n = 17], and Simmental [n = 25]) and heifers (sired by Angus [n = 25], Charolais [n = 6], Gelbvieh [n = 30], Limousin [n = 18], Red Angus [n = 14], and Simmental [n = 16]) were used in the study. Calves were housed in a facility with Calan Broadbent electronic headgates (American Calan, Inc., Northwood, NH) to measure individual feed intake. The facility consisted of soil-surfaced pens approximately one-third under a barn open to the south. The cattle were trained to use individual Calan headgates during the adaptation period (approximately 45 d). The steers were implanted (200 mg trenbolone acetate and 40 mg estradiol 17β; Revalor XS; Merck Animal Health, Summit, NJ) 86 d before the start of the experiment. Heifers received a Revalor H implant (140 mg trenbolone acetate and 14 mg estradiol; Merck Animal Health) 86 d before the beginning of the study and a Revalor 200 implant (200 mg trenbolone acetate and 20 mg estradiol; Merck Animal Health) 57 d after the study began.

Cattle were placed on a diet that, on a DM basis, consisted of 67.75% dry-rolled corn, 20% wet distillers grains with solubles, 8% chopped alfalfa hay, and 4.25% of a commercial supplement balanced to meet the minimum mineral and vitamin requirements for growth (NRC, 2000) 7 d before the feed intake and growth trial began. The supplement contained monensin to supply 300 mg/animal daily. Feed bunks were visually evaluated each day of the experiment at approximately 0730 h to determine the quantity of feed to offer each animal. The bunk management approach was designed to allow for 0.25 to 0.50 kg of feed remaining in the feed bunk at the time of evaluation. After the quantity of feed to be provided to each bunk was determined, a portion of the ration sufficient to supply the feed for all the pens was mixed in the feed truck (Roto-Mix IV 274-12B, scale readability ± 0.09 kg; Dodge City, KS) for approximately 5 min. Cattle were fed once daily throughout the experiment, starting at approximately 0800 h. Feed was subsampled daily and a weekly composite sample was made to determine feed DM. Orts were determined once per week. Dry matter intake was the sum of DM fed minus orts and divided by days on study. The feeding study lasted 84 d and cattle were weighed on d 0, 1, 21, 42, 63, 83, and 84. At the beginning of the study, heifers weighed 395 ± 3.5 kg and steers weighed 416 ± 3.5 kg. Mean age of cattle at the beginning of the study was 298 ± 0.4 d of age. A quadratic equation was used to regress BW on day of study and total BW gain was determined by solving the equation for 84 d on study. Average daily gain was calculated as total BW gain divided by days on study. The G:F was calculated as the quotient of ADG divided by daily DMI. Fractional DMI was calculated by dividing grams of daily DMI by the BW at the midpoint (**MidBW**) of the study (g DM·d^{-1}·kg MidBW^{-1}). Residual feed intake was calculated by regressing observed DMI against ADG and metabolic MidBW. Steers and heifers were treated as separate cohorts for the RFI calculation.

Following the production trait measurement period, cattle remained in the same pens with the Calan gates locked open and were fed the same ration ad libitum. Body composition was evaluated by ultrasound between d 93 and 97. Ultrasound images were collected by an

Ultrasound Guidelines Council (Miles City, MT)–certified field technician using a Classic Scanner 200 with an ASP-18 transducer (Classic Medical, Tequesta, FL). Body composition traits measured included 1) subcutaneous fat thickness over the termination point of the biceps femoris in the rump (rump fat), 2) subcutaneous fat thickness at three-fourths the lateral distance across the LM between the 12th and 13th ribs (12th rib fat thickness), 3) LM area between the 12th and 13th ribs (LM area), and 4) percent intramuscular fat (**IMF**) within the LM between the 12th and 13th ribs.

Sample Collection, Processing, and Analyses

Blood (9 mL) was collected at the end of the DMI and ADG measurement period (d 83) before feeding, via jugular venipuncture into tubes containing EDTA (1.7 μg/mL of blood) and immediately placed on ice. Samples were then centrifuged at 3,000 × *g* for 25 min at 4°C to obtain plasma. Plasma samples for cortisol analysis were stored at –20°C. Whole blood glucose and L-lactate were quantified using an immobilized enzyme system (YSI model 2700; YSI Inc., Yellow Spring, OH) within 15 min of blood sample collection. A separate aliquot of whole blood was analyzed for hematology values the same day as collection using a HemaVet CDC Mascot veterinary hematology analyzer (CDC Technologies, Oxford, CT). Samples were brought to room temperature and mixed for at least 15 min before hematology analysis. Values reported included total leukocyte count (**WBC**), neutrophil count (**NU**), lymphocyte count (**LY**), monocyte count (**MO**), eosinophil count (**EO**), red blood cell count (**RBC**), and hemoglobin concentration (**Hb**).

At the same time as the blood collection, a fecal sample was obtained via rectal grab. Fecal samples were stored at –20°C. A sample of lyophilized feces was ground through a fine mesh screen and 0.2 g was weighed for corticosterone extraction following previously published methods with slight modification (Wasser et al., 2000; Morrow et al., 2002). Five milliliters of 80% methanol was added to the dried and ground feces, and samples were mixed vigorously at room temperature for 1 h. Samples were then centrifuged at 3,000 × *g* at 6°C for 15 min. The supernatant was collected and a portion was diluted 1:5 with steroid diluent (MP Biomedicals, Irvine, CA). The diluted extract was stored at –20°C until later analysis. Extraction efficiency was tested by adding 500 μL of ^{125}I corticosterone (MP Biomedicals) to 10 dried and ground fecal samples. Five samples were from forage-fed cattle and the remaining 5 samples were from concentrate-fed cattle. Samples were extracted in duplicate as described above. Total counts of the extracts were compared with the ^{125}I corticosterone. Extraction efficiency was 95.9 ± 0.67% for the forage-fed cattle and 95.1 ± 1.22% for the concentrate-fed cattle.

Plasma cortisol was analyzed using a commercial coated-tube RIA kit (MP Biomedicals) in duplicate. The intra- and interassay CV for the cortisol analysis were 0.87 and 3.86%, respectively. Fecal corticosterone was measured in duplicate in the diluted fecal extracts using a commercial RIA kit (MP Biomedicals) with modification. A volume of 0.1 mL of 125I corticosterone tracer and sample were used, and the primary antibody was diluted 1:1 with the steroid diluent and added in a volume of 0.1 mL. After incubation for 24 h at 4°C, 0.25 mL of the precipitant solution was added. The total volume of the assay was 0.55 mL/tube. Linearity (log/logit transformation; $R^2 > 0.98$) and parallelism of the bovine fecal extract to the standard curve was determined using 7 dilutions that ranged from 2.8 to 49.9 ng/mL. When 0.025 or 0.25 ng of corticosterone was added to a test fecal extract, overall recovery was 108 ± 6.4%. Sensitivity (90% of zero standard binding) of the assay was 0.013 ng/tube. Intra- and interassay CV were evaluated using quality control samples included with the kit and were both <1.0%.

Statistical Analysis

All data were analyzed using SAS 9.3 (SAS Inst. Inc., Cary, NC). Animal was used as the experimental unit. All variables were tested for normality using the Shapiro–Wilk test in the univariate procedure. Lactate (Shapiro–Wilk test statistic [**W**] = 0.85), fecal corticosterone (W = 0.76), RBC (W = 0.88), and Hb (W = 0.86) were determined to be non-normal and were transformed to achieve near normality (W = 0.98, 0.97, 0.97, and 0.95 for transformed lactate, fecal corticosterone, RBC, and Hb, respectively). Lactate and fecal corticosterone were logarithm (base 10) transformed and RBC and Hb were squared. Transformed values were used in all analyses. Pearson correlations of variables were analyzed using the CORR procedure to describe simple associations between variables. A mixed model analysis was used to determine the association of blood metabolites, hormones, and blood cell counts with DMI, BW-adjusted DMI, ADG, G:F, and RFI while accounting for breed, sex, and other covariate effects, which cannot be accomplished with simple correlations. Interactions of variables with sex were tested and removed when not significant ($P > 0.10$). Models where the interaction with sex was removed are indicated in the data tables where only 1 solution is presented for both steers and heifers as opposed to separate solutions for steers and heifers with the interaction with sex tending to be or being significant. When the interaction was significant ($P < 0.05$) or tended to be significant ($0.10 > P > 0.05$), the Estimate statement was

used to determine the parameter for the individual sexes and if they differed from 0. In the models for DMI, ADG, and G:F, covariates included midpoint BW and breed class. Ultrasound body composition variables (LM area, 12th rib fat thickness, and rump fat thickness) were included in models only when there was an improvement in the Bayesian information criterion with their inclusion. These models included only models with individual leukocyte subtypes. Covariates were the same for BW-adjusted DMI and RFI models except that the midpoint BW covariate was removed. Covariates were included to determine the association of the metabolites, hormones, and blood cell counts with the production traits independent of BW and breed and, where appropriate, body composition. The random effect of sire was included in all models. Three sets of models (metabolites and hormones, major blood cell types and hemoglobin, and leukocyte subtypes) were used to allow for the evaluation of metabolites and hormones separate from the blood cell counts because there are potential intercorrelations between stress and immune function measures. Data presented are solutions for the mixed model analysis and are presented to demonstrate directionality and the magnitude of the association for each parameter. Two additional set of models were analyzed to determine the association of cortisol and fecal corticosterone with ultrasound body composition traits and the association of leukocyte subtypes with ultrasound body composition. Final BW was included as a covariate for this set of models.

RESULTS

Blood glucose was negatively correlated with DMI ($r = –0.24$, $P < 0.01$; Table 1), fractional DMI ($r = –0.24$, $P < 0.01$), ADG ($r = –0.26$, $P < 0.01$), and G:F ($r = –0.14$, $P < 0.05$). Blood lactate was also negatively correlated with DMI ($r = –0.28$, $P < 0.01$), fractional DMI ($r = –0.21$, $P < 0.01$), ADG ($r = –0.37$, $P < 0.01$), and G:F ($r = –0.28$, $P < 0.05$). Blood cortisol was negatively correlated to ADG ($r = –0.17$, $P < 0.01$) and G:F ($r = –0.20$, $P < 0.01$) and positively correlated to RFI ($r = 0.14$, $P = 0.03$). Fecal corticosterone was positively correlated with fractional DMI ($r = 0.15$, $P = 0.02$) and RFI ($r = 0.23$, $P < 0.01$) and negatively correlated to G:F ($r = –0.18$, $P < 0.01$). Total leukocyte count was positively correlated to all production measurements ($r \geq 0.19$, $P < 0.01$). Neutrophil count was positively correlated to DMI ($r = 0.14$, $P < 0.05$), fractional DMI ($r = 0.16$, $P < 0.05$), and ADG ($r = 0.15$, $P < 0.05$). Lymphocyte count was positively correlated to all production measurements ($r \geq 0.20$, $P < 0.01$) except RFI ($r = –0.02$, $P > 0.10$). Monocyte count was positively correlated with fractional DMI ($r = 0.14$, $P < 0.05$), ADG ($r = 0.21$, $P < 0.01$), and G:F ($r = 0.21$, $P < 0.01$). Eosinophil count was negatively correlated to DMI ($r = –0.22$, $P < 0.01$), ADG ($r = –0.30$, $P < 0.01$), and G:F ($r = –0.22$, $P < 0.01$). Red blood cell count was negatively correlated with DMI ($r = –0.17$, $P < 0.01$), fractional DMI ($r = –0.13$, $P < 0.05$), and ADG ($r = –0.18$, $P < 0.01$). Hemoglobin concentration was negatively correlated with all production measurements ($r \leq –0.22$, $P < 0.01$) except RFI ($r = 0.03$, $P > 0.10$).

Table 1. Correlation of blood metabolites, hormones, and hematological variables with productions traits in individually fed finishing beef steers and heifers of diverse genetic background ($n = 236$)

Variable	DMI	Fractional DMI[1]	ADG	G:F	RFI[2]
Glucose	–0.24**	–0.24**	–0.26**	–0.14*	–0.06
Lactate[3]	–0.28**	–0.21**	–0.37**	–0.28**	0.06
Cortisol	–0.08	–0.03	–0.17**	–0.20**	0.14*
Fecal corticosterone[3]	0.03	0.15*	–0.08	–0.18**	0.23**
Leukocyte count	0.19**	0.20**	0.25**	0.19**	0.01
Neutrophil count	0.14*	0.16*	0.15*	0.06	0.06
Lymphocyte count	0.20**	0.20**	0.30**	0.25**	–0.02
Monocyte count	0.12	0.14*	0.21**	0.21**	–0.06
Eosinophil count	–0.22**	–0.12	–0.30**	–0.22**	0.06
Red blood cell count[4]	–0.17**	–0.13*	–0.18**	–0.07	–0.03
Hemoglobin concentration[4]	–0.34**	–0.22**	–0.39**	–0.22**	0.03

[1]Fractional DMI was equal to grams DMI per kilogram BW at the midpoint per day.

[2]RFI = residual feed intake.

[3]Log base 10 transformed.

[4]Squared transformed.

*$P < 0.05$; **$P < 0.01$.

In the mixed model analysis, blood glucose concentration had a negative association with fractional DMI and RFI ($P \leq 0.05$; Table 2) and tended to have a positive association with G:F ($P < 0.10$). Blood lactate was negatively associated with ADG ($P < 0.01$). There was a sex × lactate interaction ($P = 0.02$) for G:F, indicating that lactate was more negatively associated with G:F in heifers (parameter differs from 0; $P < 0.01$) than steers (parameter tends to differ from 0; $P = 0.08$). Plasma cortisol concentrations tended to be negatively associated with ADG ($P < 0.10$) and were negatively associated with G:F ($P = 0.01$). There was a sex × fecal corticosterone interaction in the fractional DMI model ($P = 0.03$) indicating a positive association of fecal corticosterone with fractional DMI in heifers ($P < 0.01$) but not steers. Additionally, there was a sex × fecal corticosterone interaction for RFI ($P < 0.04$) indicating that in heifers, fecal corticosterone was positively associated with RFI ($P < 0.01$) but not in steers ($P = 0.60$).

Leukocyte count was positively associated with ADG ($P < 0.03$; Table 3) and G:F ($P < 0.05$). There was no association of RBC or Hb with any of the production traits ($P > 0.10$). Ultrasound body composition (12th rib

Table 2. Solutions (SEM) for the mixed model analysis of the relationship of blood metabolites and hormones with DMI, ADG, and efficiency in individually fed finishing beef steers and heifers of diverse genetic background (n = 236). Interactions with sex were excluded when not significant ($P > 0.10$)

		Lactate, log mM			Fecal corticosterone, log ng/g	
Variable[1]	Glucose, mM	Steers	Heifers	Cortisol, nM	Steers	Heifers
DMI, kg/d	–0.13 (0.101)	–0.38 (0.295)		0.0004 (0.0012)	0.54 (0.332)[a]	
Fractional DMI, g·d^{-1}·kg MidBW^{-1}	–0.42 (0.211)[b]	–0.64 (0.607)		–0.001 (0.0025)	0.01 (0.882)[d]	2.94 (1.03)[d]
ADG, kg/d	0.007 (0.027)	–0.27 (0.08)[c]		–0.001 (0.0003)[a]	–0.011 (0.0877)	
G:F, kg/kg	0.004 (0.0023)[a]	–0.01 (0.008)[e]	–0.04 (0.010)[e]	–0.0001 (0.00003)[b]	–0.010 (0.0076)	
RFI, kg/d	–0.16 (0.080)[b]	0.33 (0.231)		0.002 (0.001)	0.18 (0.34)[f]	1.25 (0.39)[f]

[a]Parameter tends to differ from 0 ($P \leq 0.10$).

[b]Parameter differs from 0 ($P \leq 0.05$).

[c]Parameter differs from 0 ($P < 0.01$).

[d]Fecal corticosterone × sex interaction, $P = 0.03$; only the heifer parameter differs from 0 ($P < 0.01$).

[e]Lactate × sex interaction, $P = 0.02$; steer parameter tends to differ from 0 ($P = 0.08$) and heifer parameter differs from 0 ($P < 0.01$).

[f]Fecal corticosterone × sex interaction, $P < 0.04$; only the heifer parameter differs from 0 ($P < 0.01$).

[1]MidBW = BW at the midpoint; RFI = residual feed intake.

fat, rump fat, and LM area) were included as covariates for models including the individual leukocyte subtype counts, as inclusion improved the Bayesian information criterion in these models alone. Neutrophil count was positively associated with ADG in steers ($P = 0.02$ for the interaction; Table 4) but not heifers. There was a LY × sex interaction for the DMI ($P < 0.01$), fractional DMI ($P < 0.01$), and ADG ($P < 0.01$) models and a tendency for an interaction ($P = 0.05$) in the G:F model. For DMI, steers had a negative association of LY ($P = 0.03$) and heifers tended to have a positive association ($P < 0.09$). The same trend was observed in the fractional DMI model, where steers had a negative association of LY with fractional DMI ($P < 0.03$) and heifers tended to have a positive association ($P < 0.06$). In the ADG model, heifers had a positive relationship ($P < 0.01$) between ADG and LY, whereas the steer relationship was not different from 0 ($P > 0.10$). Additionally, MO tended to have an interaction with sex ($P < 0.07$), with heifers having a positive relationship between MO and ADG ($P < 0.02$). Eosinophil count was not associated with any production trait ($P > 0.10$). None of the hematological measures was associated with RFI ($P > 0.10$).

Fecal corticosterone was positively associated with rump fat thickness ($P < 0.01$; Table 5). There was a sex × cortisol ($P < 0.03$) and a sex × corticosterone interaction ($P < 0.02$) for IMF. These interactions indicate a negative association of cortisol with IMF in heifers ($P = 0.02$) but not steers ($P = 0.64$) and a positive association of fecal corticosterone with IMF in heifers ($P = 0.03$) but not steers ($P = 0.22$). Neutrophil ($P < 0.05$) and MO ($P < 0.04$) were negatively associated with 12th rib fat thickness measured by ultrasound. Eosinophil counts tended ($P < 0.06$) to be negatively associated with 12th rib fat thickness and LY tended ($P < 0.06$) to be positively associated with 12th rib fat thickness. Eosinophil counts were positively associated ($P < 0.01$) with LM area. Lymphocyte counts tended ($P < 0.10$) to be positively associated with IMF content.

DISCUSSION

The association of glucocorticoids with feed efficiency and the component traits has not been extensively researched in cattle. Cortisol is the major glucocorticoid produced in cattle in response to ACTH, which increases in response to stress (Minton, 1994). Cortisol is constantly released into circulation, but acute stressors quickly increase cortisol concentrations in blood (von Holst, 1998). Factors other than stress can have effects on circulating glucocorticoid concentrations. For instance, cortisol concentrations have a diurnal

Table 3. Solutions (SEM) for the mixed model analysis of the relationship of total leukocyte count, red blood cell count, and hemoglobin concentration with DMI, ADG, and efficiency in individually fed finishing beef steers and heifers of diverse genetic background (n = 236). Interactions with sex were excluded when not significant ($P > 0.10$)

Variable[1]	Leukocytes count, cells × 10^3/μL	Red blood cells, (cells × 10^6/μL)[2]	Hemoglobin, (g/dL)[2]
DMI, kg/d	0.02 (0.052)	–0.002 (0.0067)	–0.0005 (0.0041)
Fractional DMI, g·d^{-1}·kg MidBW^{-1}	0.03 (0.11)	–0.003 (0.014)	–0.004 (0.0085)
ADG, kg/d	0.03 (0.014)[a]	0.0002 (0.0018)	–0.001 (0.0011)
G:F, kg/kg	0.002 (0.0012)[b]	0.0001 (0.00016)	–0.0001 (0.00010)
RFI, kg/d	–0.04 (0.041)	–0.005 (0.0053)	0.004 (0.0033)

[a]Parameter differs from 0 ($P < 0.03$).

[b]Parameter differs from 0 ($P < 0.05$).

[1]MidBW = BW at the midpoint; RFI = residual feed intake.

Table 4. Solutions (SEM) for the mixed model analysis of the relationship of neutrophil count (NU), lymphocyte count (LY), monocyte count (MO), and eosinophil count (EO) with DMI, ADG, and efficiency in individually fed finishing beef steers and heifers of diverse genetic background (n = 236). Interactions with sex were excluded when not significant ($P > 0.10$)

	NU, cells × 10^3/μL		LY, cells × 10^3/μL		MO, cells × 10^3/μL		
Variable[1]	Steers	Heifers	Steers	Heifers	Steers	Heifers	EO, log k/μL
DMI, kg/d	0.081 (0.099)		–0.22 (0.103)[a]	0.23 (0.133)[a]	0.42 (0.89)		0.037 (0.189)
BW-adjusted DMI, g·d^{-1}·kg MidBW^{-1}	0.13 (0.208)		–0.48 (0.21)[b]	0.53 (0.27)[b]	1.39 (1.86)		0.21 (0.39)
ADG, kg/d	0.089 (0.034)[c]	–0.04 (0.029)[c]	–0.046 (0.029)[d]	0.12 (0.038)[d]	–0.007 (0.300)[e]	0.95 (0.42)[e]	–0.047 (0.052)
G:F, kg/kg	0.002 (0.00026)		–0.0005 (0.0026)[f]	0.007 (0.003)[f]	0.02 (0.023)		–0.005 (0.0048)
RFI, kg/d	0.028 (0.083)		–0.098 (0.071)		–0.61 (0.731)		0.09 (0.157)

[a]LY × sex interaction, $P < 0.01$; steer estimate differ from 0 ($P = 0.03$) and heifer estimate tends to differ from 0 ($P < 0.09$).

[b]LY × sex interaction, $P < 0.01$; steer parameter differs from 0 ($P = 0.03$) and heifer parameter tends to differ from 0 ($P < 0.06$).

[c]NU × sex interaction, $P = 0.02$; only the parameter for the steer model differs from 0 ($P = 0.02$).

[d]LY × sex interaction, $P < 0.01$; only the heifer parameter differs from 0 ($P < 0.01$).

[e]MO × sex interaction, $P < 0.07$; only the heifer parameter differs from 0 ($P < 0.03$).

[f]LY × sex interaction, $P = 0.05$; only the heifer parameter differs from 0 ($P = 0.03$).

[1]MidBW = BW at the midpoint; RFI = residual feed intake.

pattern (Thun et al., 1981) but variation throughout the day is likely less in ad libitum fed cattle, such as the cattle in the current experiment, compared with meal-fed or nutrient-restricted cattle (Sejrsen et al., 1983). The only stress inflicted on the cattle in this experiment was processing cattle to collect BW measurements and biological samples. It is not possible to determine what factors are contributing to the observed glucocorticoid concentrations, but it is obvious that a large amount of variation was observed in this study. Cortisol concentrations ranged from 6.2 to 283.8 nM with a mean (SEM) of 83.7 nM (3.05). Fecal corticosterone concentrations ranged from 6.4 to 87.2 ng/g of fecal DM with a mean of 21.0 ng/g fecal DM (SEM 0.6). The divergence in variation magnitude between fecal corticosterone and plasma cortisol could indicate that weighing and collecting biological samples from the cattle induced a stress response in some cattle.

Recent efforts have focused on discovering parameters to quantify either long-term stress or measures that themselves do not introduce stress to the animals and, therefore, increase glucocorticoid concentrations. Fecal glucocorticoid metabolites and corticosterone is one such method, as they are representative of glucocorticoid concentrations 12 to 18 h before sample collection and are responsive to stressors such as transport, new environments, and ACTH challenge (Morrow et al., 2002). The 11,17-dioxoandrostanes (**DOA**) are the main metabolites of cortisol that have been measured in feces of livestock (Mostl et al., 1999). The assay commonly used measures several metabolites of the steroid base that have the side chain removed and an oxygen molecule on the 11-carbon and 17-carbon of the steroid moiety. Corticosterone is another glucocorticoid that is produced in response to ACTH release (Morrow et al., 2002), in a pattern similar to cortisol. Additionally, appearance of corticosterone in feces is similar to the pattern of appearance of the primary cortisol metabolites and is stable during storage and processing (Morrow et al., 2002). As corticosterone is representative of adrenal activity, can be measured using a commercially available RIA kit, and is relatively stable in fecal samples, it was chosen as one measure of glucocorticoids that would likely represent adrenal activity 12 to 18 h before processing the cattle. In addition to the fecal corticosterone measurement, a single time-point measure of plasma cortisol was included in this study.

Fecal corticosterone was shown to be positively correlated with RFI, and the mixed model analysis indicated that fecal corticosterone is positively associated with RFI in heifers but not steers. These data indicate that more efficient heifers (low RFI) have lower concentrations of fecal corticosterone than the high-RFI heifers. Two previous studies aimed to determine the association of a glucocorticoid metabolite (DOA) in feces to RFI (Montanholi et al., 2010, 2013). The assumption of the approach taken by Montanholi et al. (2010, 2013) is similar to the assumption of the approach taken in the present study; that is, the concentration of either DOA or corticosterone in the feces is representative of adrenal activity 12 to 18 h before the fecal sample collection. There is no data available to indicate that the 2 approaches are comparable with each other, but it is noteworthy that the conclusions of the 2 previous studies (Montanholi et al., 2010, 2013) and the present study are opposite in regards to RFI. The difference could be due to a number of factors including the assays used or the population of cattle used.

Table 5. Solutions (SEM) for the mixed model analysis of the relationship of ultrasound measured body composition with plasma cortisol and fecal corticosterone or individual leukocyte subtype counts in individually fed finishing beef steers and heifers of diverse genetic background (n = 236). Body weight on day of measurement was included as a covariate. Interactions with sex were excluded when not significant ($P > 0.10$)

	Cortisol		Fecal corticosterone					
Variable	Steers	Heifers	Steers	Heifers	NU[1]	LY[1]	MO[1]	EO[1]
12th rib fat	0.001 (0.001)		0.15 (0.13)		–0.079 (0.039)[b]	0.07 (0.034)[a]	–0.73 (0.35)[b]	–0.13 (0.067)[a]
Rump fat	0.001 (0.001)		0.31 (0.11)[b]		–0.003 (0.034)	0.03 (0.030)	–0.49 (0.306)	–0.06 (0.059)
LM area	0.016 (0.012)		2.51 (3.54)		–0.33 (1.07)	0.10 (0.925)	–13.2 (9.49)	4.88 (1.826)[b]
Intramuscular fat	0.001 (0.001)[c]	–0.004 (0.0019)[c]	–0.64 (0.54)[d]	1.16 (0.537)[d]	–0.18 (0.108)	0.16 (0.094)[a]	1.12 (0.966)	–0.046 (0.1855)

[a]Parameter tends to differ from 0 ($P < 0.10$).

[b]Parameter differs from 0 ($P < 0.05$).

[c]Cortisol × sex interaction, $P < 0.03$; only the heifer parameter differs from 0 ($P = 0.02$).

[d]Fecal corticosterone × sex interaction, $P < 0.02$; only the heifer parameter differs from 0 ($P = 0.03$).

[1]NU = neutrophil count; LY = lymphocyte count; MO = monocyte count; EO = eosinophil count.

Long-term elevation of glucocorticoid concentrations leads to major changes in glucose and AA metabolism, such as increased protein catabolism in myocytes (Baxter and Forsham, 1972), which could have negative effects on feed efficiency in cattle. It is likely that short-term increases in glucocorticoids can lead to the same shifts in metabolism but may not be as dramatic or long lasting as chronic elevation, as the main mechanisms of glucocorticoids are alterations in gene expression and protein synthesis (Baxter and Forsham, 1972). Therefore, the lack of an association of the fecal glucocorticoid measure in the current data suggest that the metabolic effects of the glucocorticoids are likely not as important as the acute cortisol measure. If the negative metabolic effects of glucocorticoids were important for feed efficiency, then either high ADG or high G:F would likely be associated with low fecal corticosterone. This effect was observed when using RFI as a measure of feed efficiency in heifers, which could indicate that low RFI corresponds with fewer negative effects of cortisol on nutrient metabolism. However, the data presented here is in disagreement with previous data that showed high chronic glucocorticoid exposure in low-RFI cattle (Montanholi et al., 2010, 2013). This could indicate that not all low-RFI cattle have altered glucocorticoid biology.

Although chronic stress can have detrimental effects on production and feed efficiency (Purchas et al., 1980), the nature of an animal's response to acute stressors can also have a large effect on feed efficiency. In sheep displaying divergent RFI, it was shown that less efficient sheep (high RFI) had a greater response to an ACTH challenge than the low-RFI sheep (Knott et al., 2008, 2010). Data presented by Knott et al. (2008) also showed that basal cortisol concentrations were less associated with feed efficiency than either the post-ACTH challenge cortisol concentrations or the change in cortisol concentrations. Similarities can be drawn between the concept of an association of the response to an ACTH challenge to feed efficiency and data in the present study indicating an association of plasma cortisol during a stressful event (handling and bleeding the cattle) with growth and feed efficiency. Although the current study did not set out to measure the stress response in cattle, the similarities between a single cortisol measurement from a large number of cattle during a stress event and an intensive measure of a stress response in a small set of animals is noteworthy. Further research is needed to determine if the similarities between large experiments with a single measure and intensive measures on a small scale are repeatable. It is also possible that the cortisol concentrations measured are not actually in response to stress but simply represent natural variation in cattle.

Data presented here indicate that plasma cortisol concentrations are associated with growth and feed efficiency (G:F) whereas fecal corticosterone concentrations are not associated with actual efficiency. This is complemented with the negative association of blood lactate concentrations with growth and G:F (in heifers), as lactate is positively associated with certain acute stressors (Mitchell et al., 1988) or could be the result of altered glucose metabolism due to cortisol actions. The results were slightly different when using RFI as a measure of feed efficiency, where fecal corticosterone was positively associated with RFI in heifers but not steers; that is, more efficient heifers displayed lower fecal corticosterone. These data indicate that heifers displaying low RFI have lower concentrations of glucocorticoids in the feedlot. This association is unique to RFI in heifers in this experiment, as the relationship of RFI and fecal corticosterone was not identified in steers. The difference in associations between fecal corticosterone and RFI in steers and heifers could indicate that the biology of RFI is different in steers and heifers.

Hematology measures are used to evaluate overall health and aid in diagnosis of diseases. There is no doubt that animal health will have a large effect on feed efficiency, as a sick animal will not achieve the same level of production as a healthy animal (Johnson, 1997; Spurlock, 1997). However, it is not clear if subclinical disease or small changes in blood cell counts is associated with production efficiency. The association of hematological parameters with production traits and feed efficiency has not been heavily studied. One of the few published experiments that studied the association of blood cell counts with feed efficiency used steer progeny from sires selected for either high or low RFI (Richardson et al., 2002). It was shown that there was a negative association with sire EBV for RFI with WBC and LY. Assuming the EBV for RFI was representative of the actual feed efficiency of the steer progeny, data presented here, where WBC was positively associated with G:F, is in agreement with the previous report (Richardson et al., 2002). Another study showed that rams with low RFI have lesser WBC than high-RFI rams but not ewes (Rincon-Delgado et al., 2011). Additionally, in the present study, LY was positively associated with G:F in heifers but not in steers, which is similar to a previous report (Richardson et al., 2002). Similar to results from this study, studies conducted with Nellore steers and bulls showed no association of blood cell counts with RFI (Gomes et al., 2011; Santana et al., 2013) and a study using rams showed no association between RFI and WBC (Paula et al., 2013). No previous reports could be found relating WBC with DMI or ADG.

Red blood cells and Hb are important for oxygen delivery to tissues and could potentially limit oxidative metabolism if concentrations are low enough to reduce the amount of oxygen delivered to tissues with high metabolic demands. Richardson et al. (2002) showed a slight positive association of Hb with sires' EBV for RFI, indicating that steers with the genetic potential for low RFI have slightly lower Hb than steers with high RFI potential. The same association of RBC with RFI was observed in rams in one study (Rincon-Delgado et al., 2011) but was not observed in another study with rams (Paula et al., 2013). Studies with Nellore steers and bulls showed no association of RBC or Hb with either RFI or feed conversion ratio (Gomes et al., 2011; Santana et al., 2013). No previously reported studies could be found relating RBC and Hb with DMI or ADG. In the current study, RBC and Hb are not significantly associated with production traits in finishing beef cattle.

A link between obesity and immune function has been observed in humans and rodent models (Martí et al., 2001; McNelis and Olefsky, 2014). This link between immune function and body fatness has not been studied in livestock and specifically cattle. However, it is likely that the mechanisms present in rodents and humans will be similar in ruminants, although the definition and characteristics of obesity may be dissimilar. Data presented here show a potential link between body fatness and impaired immune function, although there is no indication of the direction of causality or if there are indirect effects on the 2 factors. Reduced numbers of NU, MO, and EO and slightly increased numbers of LY was associated with increased 12th rib fat thickness measured by ultrasound. This trend is not entirely similar to human data, as obesity in humans has been associated with higher basal levels of WBC, NU, LY, and MO, yet obese humans had impaired mitogen-induced LY proliferation (Nieman et al., 1999). This indicates that even though obese subjects have greater numbers of WBC, the function of those cells are likely impaired, making the subjects more susceptible to disease. This trend is similar to the data reported here, where increases in 12th rib fat thickness is associated with a reduced number of NU, MO, and EO and could be related to impaired immune function. This observation may be of minimal importance for finishing cattle, as most production settings involve few introductions of new disease challenges during the final stages of production where 12th rib fat thickness is greatest. This could, however, impact younger cattle that have greater body fatness at times of feedlot entry and commingling.

In conclusion, data presented here provide further indication that both fecal corticosterone and plasma cortisol are associated with production traits and are likely good indicators for production traits and feed efficiency in finishing beef cattle. Additionally, hematological parameters could be useful as physiological markers for productions traits but would be dependent on sex.

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